ALASKA OREGON WASHINGTON
PACIFIC NORTHWEST FOREST AND RANGE EXPERIMENT STATION

USDA FOREST SERVICE RESEARCH NOTE

PNW-265 December 1975

IMPROVED LABORATORY METHODS FOR TESTING GRAFT COMPATIBILITY IN DOUGLAS-FIR

by

Charles E. Gnose, *Forestry Technician*

and

Donald L. Copes, *Principal Plant Geneticist*

ABSTRACT

Improved laboratory methods for preparing microscope slides from Douglas-fir graft unions are presented. Descriptions of equipment and supplies and graft collection, sectioning, and staining methods are all outlined in detail. Sections from unembedded graft unions are cut with a sliding microtome. A number of modifications of the traditional safranin and fast green staining method are made to obtain the best results in the shortest time. The cut sections are placed in specially designed staining baskets and transferred through a 14- or 18-step staining schedule. Solution concentrations, the time sections are left in each solution, as well as methods for correcting under- and over-staining, are listed. Procedures for preparing both temporary and permanent slides are described. The methods are fast and accurate. Excellent staining can be achieved by technicians with little or no formal training. The methods also work well for many other conifers and are effective on normal stems as well as graft unions.

KEYWORDS: Grafting, Douglas-fir.

INTRODUCTION

Graft incompatibility has hindered Douglas-fir orchardists for a number of years[1/] and will continue to be a problem until highly graft-compatible rootstocks are available for general use. Simplified laboratory methods for detecting incompatibility in Douglas-fir grafts were reported,[2/] but the methods were not very efficient when hundreds or thousands of unions were tested.

Quality, accuracy, reproducibility, speed, and simplicity are requirements for efficient laboratory methods when many slides must be made. After 10 years of graft testing at the Corvallis laboratory, we have developed such methods. Descriptions of these methods follow, written in detail so that people unfamiliar with microtechnique procedures, equipment, and supplies can produce satisfactory results without additional information or training. Graft collection, microtome requirements and adjustments, staining equipment and solutions, sectioning and staining techniques, and mounting equipment and materials are discussed in separate sections.

Graft Collection Techniques

Graft unions are collected in late summer or early fall of the year after grafting (15-18 months). Collection after the cambium has ceased growth is best because the bark is tightly attached to the stem. Bark retention on stained sections is necessary for compatibility analysis of some species. On Douglas-fir grafts it is helpful but not necessary. Douglas-fir grafts that are collected in the spring before cambial growth starts the second year do not have the anatomical structures required to indicate whether the grafts are compatible or incompatible and are worthless for test purposes.

The unions should be severed from the stock and scion by cuts approximately 1 cm above and 1 cm below the graft union (fig. 1A). Sharp scissor-type pruning shears are best for severing unions. The severed union should be between 3 and 5 cm long. When field collectors become familiar with union anatomy, laboratory preparation time can be reduced by cutting through the area of the union where sections will be made (fig. 1B). The upper cut should be made at a right angle to the long axis of the stem. This cut is usually the area of the least grafting technique deformity and is usually the smoothest area of the union. If the stock and scion were both the same diameter when grafted, the top cut is made where stock and scion match on both sides of the stem. The lower cut, which severs the graft union from the stock, is made approximately 1 cm below the union. It is diagonal to the long axis of the stem, allowing the technician to quickly identify the end of the graft from which sections are not to be made.

After the unions are cut from the stock and scion, they are placed into 8- to 30-ml shell vials or small containers filled with a preservative solution of 30- to 50-percent alcohol. Alcohol concentrations above 50-percent should not be used because the unions become too hard for easy microtome sectioning. If unions are accidentally preserved in alcohol solutions that cause the tissues to harden, they can be softened before sectioning by overnight soaking in water. Either ethanol or isopropanol can be used as a preservative.

[1/] Copes, D. L. 1970. Initiation and development of graft compatibility symptoms in Douglas-fir. Silvae Genet. 19(2-3): 101-107.

[2/] Copes, D. L. 1967. A simple method for detecting incompatibility in 2-year-old grafts of Douglas-fir. U.S. Dep. Agric. For. Serv. Res. Note PNW-70, 8 p. Pac. Northwest For. and Range Exp. Stn., Portland, Oreg.

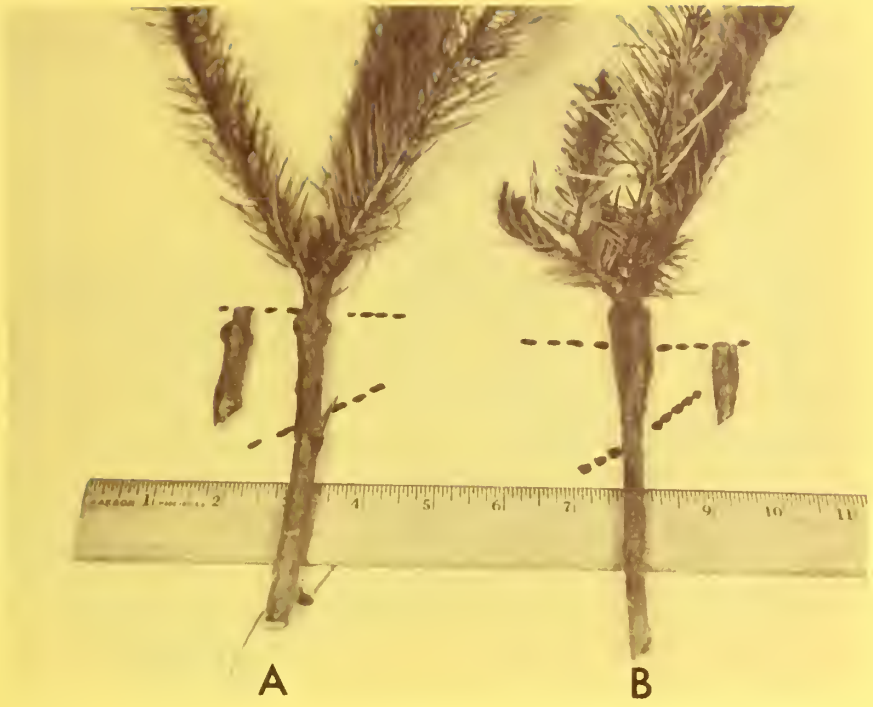


Figure 1.--Two methods of severing grafts from the root-stock and scion. Graft A is cut slightly above and below the union. In graft B the top cut is made through the area of the union where sections are to be sliced. A ruler calibrated in inches is shown for scale.

Grafts should remain in the alcohol solution at least 24 hours before sectioning and can be stored in the alcohol indefinitely. Each container should be clearly marked with the scion clone number and stock number.

Microtome Requirements and Adjustments

A sliding microtome should be used rather than attempting to slice free-hand sections with a knife or razor blade. Rotary microtomes are also unsatisfactory. One example of a satisfactory microtome is shown in figure 2. The sliding microtome should have an automatic advancement (feed) mechanism and a knife clamp that is large enough to hold a 200- to 250-mm knife. Large knives are better than small ones because more grafts can be cut before resharpening is needed.

To cut good sections a sharp knife is essential. One indicator of a dull cutting edge is alternate thin and thick sections, although this can occur if the tilt angle adjustment allows the knife to drag across the surface of the graft on the return stroke. Another indicator of a dull knife is sections with ragged and torn bark tissues. Approximately 35 to 100 grafts can be cut on each 2.5 cm of knife edge. The actual number depends upon graft size and the alcohol concentration used as the preservative. Knives should be sharpened when the entire cutting edge is dull. Sharpening should be done by a commercial or medical knife sharpening company.

Correct positioning of the knife in the microtome is important. The vertical (clearance or tilt) angle of the knife should be adjusted so that

Figure 2.--One type of suitable sliding microtome. A 250-mm knife is clamped into a heavy-duty microtome knife holder.

it is as flat as possible. The top of the graft should not touch on the lower surface of the knife when the knife is returned to the forward cutting position. The cutting edge of the knife and the knife holder should be so oriented that the knife edge meets the graft at a 60° horizontal (slice) angle. Trial and error testing will help determine which vertical and horizontal angles work best. Sections of poor quality are produced when improper angle adjustments are used. The correct angles should be checked after a knife is sharpened, as incorrect sharpening sometimes changes the angles that work best.

The base of the microtome closest to the technician should be elevated about 2.5 cm higher than the far end. Elevating will cause the knife to automatically slide away from the operator and specimen and greatly reduces the chance of technicians accidentally getting cut on the exposed knife edge.

The specimen advancement (feed) mechanism should be set to cut sections 20 to 26 um thick. Such sections are thin enough for easy microscope viewing, yet thick enough to permit them to be transferred without damage from the last staining baskets to microscope slides.

Staining Equipment and Solutions

Nine staining baskets should be constructed from small-mesh wire screen (fig. 3). Screen sizes 16 by 18 mesh or 1/8-in are both satisfactory. Copper screen is best because it does not react with the chemicals used in staining. Galvanized screen is suitable if copper is not available. The wire must be heavy enough to retain its shape when bent into the correct form.

Figure 3.--Materials and equipment needed to fabricate staining baskets. The sides of the baskets are formed by bending the circular piece of wire screen over a bottle (8-cm diameter). The wire strips that make the inner dividers are shown on the lower left. A ruler (cm) is shown for scale.

The staining baskets are designed to fit into the bottom half of a petri dish. They are circular, approximately 8 cm in diameter and 1 cm high. The main body (bottom and sides) of each basket is made from a single piece of screen 10 cm in diameter (fig. 3). The sides of the baskets are made approximately 1 cm high by bending the screen over the end of a circular disk, tube, or bottle that is slightly smaller than the inside diameter of the bottom half of a petri dish. The interior of each molded basket is divided into four compartments by soldering one 8- by 1-cm and two 4- by 1-cm pieces of screen into position (fig. 3). The dividing strips must be carefully soldered to the bottom and sides of the basket or the thin graft sections might slip under the dividers and become mixed with sections from other grafts in adjacent compartments.

The following materials are used in the staining and sectioning processes: one medium-sized camel's-hair brush; one cover-glass forceps with flat, bent ends; one pointed-tip forceps; a small syringe; one roll of heavy-duty aluminum foil; a timing clock; and two sponges or absorbent paper towels.

The table or bench top where sectioning and staining are done should be covered for protection. Heavy paper effectively prevents damage from spilled water and chemicals and can be replaced when necessary.

Glass containers should be used for staining solutions because some of the solutions will dissolve plastics and other materials. Three petri dishes are filled with water and placed to the left side of the microtome (fig. 4). On the right

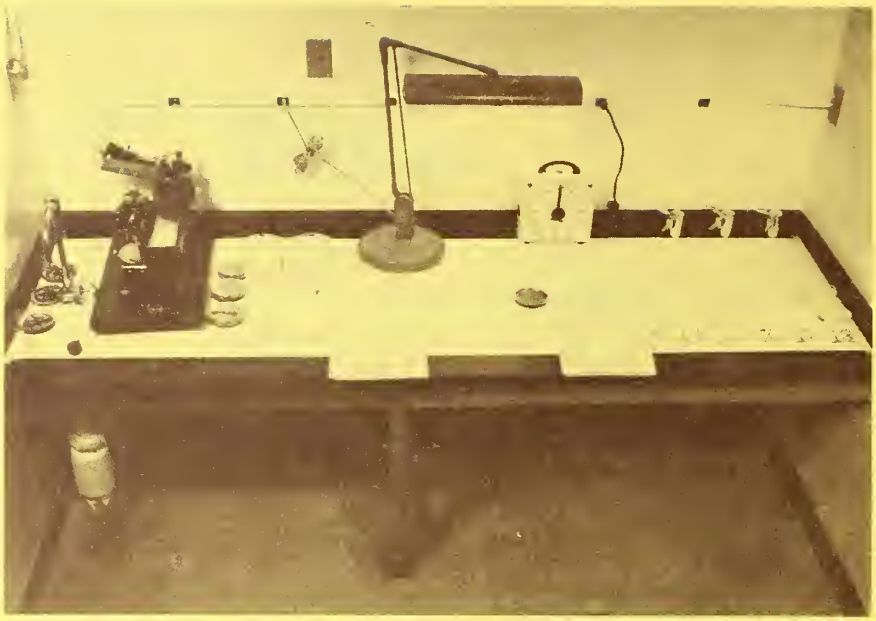


Figure 4.--Laboratory organized for a right-handed technician. The numbers correspond to the step numbers in figure 5.

side of the microtome 23 dishes and 2 beakers or jars are placed in the correct order and filled with the proper solutions (fig. 4). If a person is left-handed, the position of the microtome and dishes should be reversed.

Petri dishes should be filled with approximately 50 ml of solution. The upper edges of the baskets and the compartment dividers should always be above the top level of the solutions. If too much solution is used, sections will float between compartments and graft identity will be confused. Alcohol rapidly evaporates when left uncovered, so the open petri dishes should be covered when not in use. Periodic refilling and solution changes are required to replace evaporated liquid and to eliminate contaminated or dirty solutions.

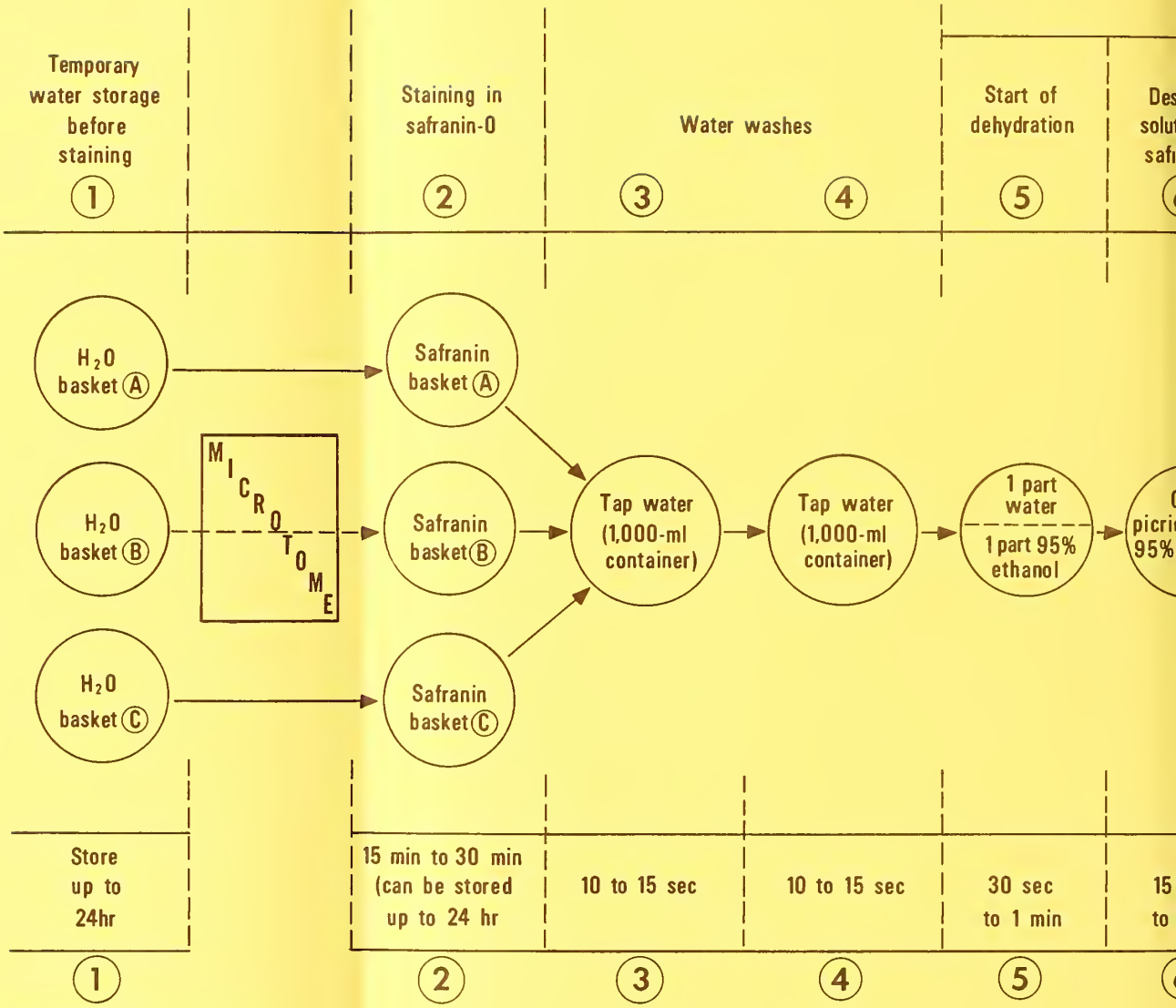
The first three petri dishes on the right side of the microtome (step 2, fig. 5) are filled with a 1-percent safranin solution in water (1 g safranin-0 in 100 ml H_2O). Additional safranin solution should be prepared and stored until needed.

The next two containers (steps 3 and 4) are 1000-ml beakers or large jars filled with tap water. The water is used to rinse excess safranin stain from the baskets and sections and must be changed whenever it becomes darkly colored or the excess stain will not be removed. The next petri dish is filled with a 1:1 solution of water and 95-percent ethanol (step 5). The following dish contains a 0.5-percent solution of picric acid in 95-percent ethanol (0.5 g of picric acid in 100 ml of 95-percent ethanol) (step 6). Picric acid rapidly removes loosely bound safranin from cell areas where it is not desired.[3/] Other acids can

---

[3/] Johansen, D. A. 1940. Staining procedures - safranin and combinations. In Plant microtechnique, p. 80-82. McGraw-Hill Book Co., Inc., New York and London.

Figure 5.--Diagram of proper seque



TIMING SCHEDULE

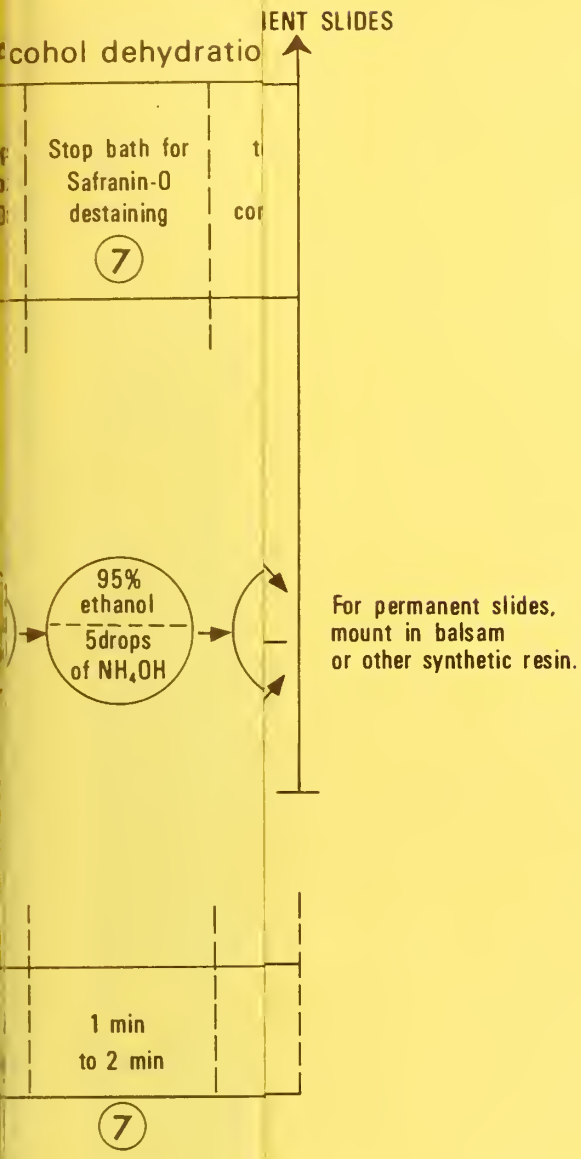
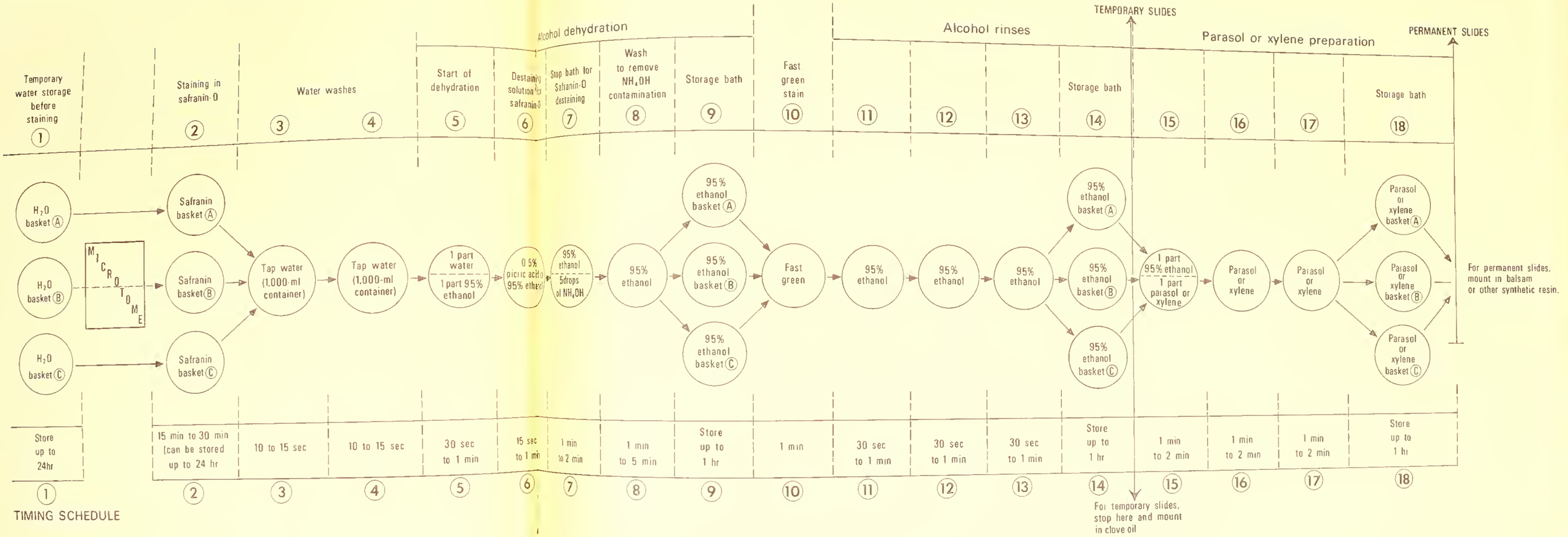

IENT SLIDES
cohol dehydratio
Stop bath for
Safranin-O
destaining
7
95%
ethanol
5drops
of NH4OH
For permanent slides,
mount in balsam
or other synthetic resin.
1 min
to 2 min
7

Figure 5.--Diagram of proper sequence and positioning of staining dishes, dish contents, time in each dish, and purpose of each step. The dishes are arranged for a right-handed technician.



be substituted for picric acid, but the technician must determine the correct concentration for each.

The next petri dish contains 50 ml of 95-percent ethanol and five drops of concentrated ammonia hydroxide (NH_4OH) (step 7). To maintain the proper solution level in the petri dish, additional NH_4OH solution can be prepared by mixing 100 ml of 95-percent ethanol and 10 drops of NH_4OH. The NH_4OH stops the destaining action that was started by the picric acid in step 6. The following petri dish contains 95-percent alcohol (step 8). The alcohol dilutes or washes off any NH_4OH transferred as contamination from the previous petri dish. The next three petri dishes are filled with 95-percent ethanol (step 9) and used as temporary storage or holding dishes until additional baskets are moved forward. Each basket is placed into only one of these dishes.

Fast green staining solution is put into the next dish (step 10). Best results are usually obtained with the following recipe: 100 ml 95-percent ethanol, 100 ml clove oil, 100 ml methyl cellosolve (2-methoxyethanol), and 0.5 g of fast green FCF (see footnote 3). If methyl cellosolve is not available, use the following recipe: 100 ml 95-percent ethanol, 100 ml clove oil, and 0.5 g of fast green FCF. If both clove oil and methyl cellosolve are unavailable, use 0.5 g of fast green FCF per 100 ml of 95-percent ethanol. Fast green stain should be prepared at least 1 day before use.

The six dishes following the fast green are filled with 95-percent ethanol (steps 11-14). Their purpose is to wash off excess fast green from the sections and baskets before the sections are mounted on microscope slides. The staining schedule ends with step 14 when temporary microscope slides are wanted.

For permanent slides, steps 15 through 18 are added. The first dish (step 15) is filled with a 1:1 solution of 95-percent ethanol and parasol (Scientific Products Co.)[4/] or xylene. The remaining dishes are filled with undiluted parasol or xylene (steps 16-18). The three dishes in step 18 are used to store the stained sections temporarily until they can be permanently mounted on microscope slides. Parasol is preferred over xylene because it is less toxic. Xylene can cause health problems after prolonged use in poorly ventilated rooms.

Sectioning Techniques

Each graft container must be given a laboratory identification number in addition to the scion and stock number marked in the field. The best technique is to write the identification number on the top of the corks or caps covering each container. Corresponding identification numbers are written in a vertical column on a 1-cm-wide strip of aluminum foil. Each number is then cut from the strip and is put into an empty basket compartment sequentially in the order that the grafts are to be sectioned. To start the procedure, three baskets are put into the dishes of water (step 1, fig. 5).

At this stage the technician is ready to begin sectioning. The first graft should be removed from its vial and trimmed through the proper union area. If it was cut through the graft union when collected (fig. 1B), no further trimming is necessary. A good quality scissor-type (not the anvil type) pruning shears or a razor blade is used to expose the area of the graft where the best match of stock and scion tissue exists. A low power hand lens or battery-powered magnifier insures that the correct union area is exposed. After trimming,

---

[4/] Mention of company by name does not imply endorsement by U.S. Department of Agriculture.

the graft is tightened securely in the specimen clamp of the microtome so that the graft will not vibrate when the knife cuts the union. When a graft has a union of stock and scion tissues on only one side of the stem, the graft is turned so that the best union surface faces the knife. Proper graft positioning in relation to the knife reduces splitting and tearing and results in better cut sections.

A rubber syringe filled with tapwater is used to put several drops on top of the graft and several more drops on the edge of the microtome knife where sections are to be cut. Wetting the two surfaces allows the sections to float on the blade when they are cut and prevents them from tearing or rolling-up. The cut section is removed from the knife with a fingertip or a small camel's-hair brush before the next section is cut. The sections are placed in the staining basket compartment labeled with the same number as the vial from which the graft was taken. Four to six good sections should be cut from each graft. When these procedures have been repeated with three more grafts, all four compartments of basket A (step 1) contain cross-sections. Sectioning is continued with eight additional grafts until staining baskets B and C are completed (step 1). The three filled baskets are then blotted on the first sponge to remove excess water and are put into the petri dishes containing safranin stain (step 2). While the sections in the first three baskets are being stained, three more baskets are placed in the dishes of water on the left side of the microtome, and 12 more grafts are sectioned. This same progressive method of cutting and staining is repeated throughout the workday.

One helpful technique for difficult grafts is to place a 2.5-cm-wide strip of wet paper towel on the upper surface of the specimen before each cut is made. The paper will help prevent sections from rolling-up and, since the section will stick to the paper, the paper can be used to transfer the section from the specimen to the staining basket.

Staining Procedures

Three staining baskets (A, B, and C) should be transferred in sequence through the different dishes of the staining schedule (fig. 5). Working with three baskets at a time is efficient for one person. However, if two people are working together, six staining baskets (24 grafts) in a series is efficient. When six baskets are used, three additional petri dishes must be added to each of steps 2, 9, 14, and 18.

Staining baskets must be blotted on sponges or absorbent paper towels before being placed into the next dish. Blotting is important because excess fluid inadvertently transferred between dishes causes contamination which will reduce quality and make frequent solution changes necessary. When sponges rather than absorbent paper towels are used, the first sponge is used for blotting all basket transfers before the dish containing fast green. The second sponge is used for the dish containing fast green and all following dishes. Sponges must be washed in hot water at the end of each day.

Sections should be left in the safranin solution at least 15 to 30 minutes (step 2). Staining times of 1 to 2 hours produce slides with greater green and red contrast. Sections can be left overnight in the safranin stain if necessary. Basket A should be removed from the safranin dish and washed in the first container of water (step 3). Washing is repeated in the next dish (step 4). The basket is then moved into the water-alcohol solution where it should remain from 30 to 60 seconds (step 5). The basket is then placed into

the destaining solution of picric acid (step 6), for 15 seconds to 1 minute. If sections have been left overnight in the safranin stain, the picric acid destaining time must be increased to approximately 2 minutes or until adequate destaining has occurred. The exact time will vary according to how long the sections are left in the safranin, strength of the safranin solution, and thickness of the sections. A longer interval in the picric acid solution is indicated if safranin continues to wash out of sections and discolors the alcohol solutions in step 9. After destaining, basket A is placed into the petri dish containing alcohol and NH_4OH (step 7) for 1 to 2 minutes and then transferred to the next petri dish (step 8). It should remain in the alcohol solution for 1 to 5 minutes and then be placed into storage dish A (step 9). Baskets B and C are advanced in the same manner and are stored in dishes B and C, respectively, of step 9. Sections can remain in 95-percent alcohol solutions of step 9 for up to 1 hour without damage, but prolonged storage slowly causes the tissues to become hard and brittle.

After baskets A, B, and C are advanced to step 9, basket A is placed in the dish containing fast green (step 10). The basket should be agitated up and down several times while it is in this stain. Agitation separates overlapping sections and insures uniform staining. The basket is left in this stain for approximately 1 minute. The time that sections are in the fast green must be carefully controlled. Staining solutions become weak after continued use. Adding additional fresh stain solution to compensate for that carried out on sections and baskets normally keeps the dish at satisfactory strength. The exact time of staining in fast green will vary with section thickness, length of time the sections were left in safranin, length of time of destaining in picric acid, and the concentration of the fast green solution.

If sections under- or over-stain with fast green, correct staining can be obtained by lengthening or shortening, respectively, the time that the sections are submerged in the solution. If proper staining is still not obtained, the concentration of fast green can be either increased or decreased. Changing the time is preferred to changing the solution concentration. Sections stained in safranin 15 to 30 minutes are left in fast green for 1 minute. Thick sections and sections that have been left in safranin overnight should be stained in fast green an additional minute.

Basket A is removed from the fast green dish (step 10), blotted on the second sponge, and placed into the first petri dish of 95-percent ethanol (step 11). The basket should be agitated up and down in the alcohol wash for 4 or 5 seconds to rapidly remove excess stain and prohibit further staining. The basket should remain in step 11 for approximately 1 minute. While basket A is in the first alcohol rinse, basket B is placed into the fast green staining dish (step 10), and basket C is removed from the ethanol storage bath (step 9) and placed into the fast green when basket B is removed. Baskets A, B, and C are moved through the remaining dishes in this manner. About 1 minute in each alcohol rinse (steps 11-13) is satisfactory, but this time can be lengthened without loss of quality. If the sections are not stained dark enough, they can be transferred in the reverse direction through the alcohol sequence and restained in fast green. Sections overstained in fast green cannot be destained.

For temporary slides, the baskets are placed into the A, B, and C storage dishes of 95-percent ethanol (step 14), and sections are trans-

ferred from the baskets to microscope slides.

If permanent slides are to be made, four more steps and six more petri dishes are needed (steps 15-18). The first dish (step 15) contains a 1:1 solution of 95-percent ethanol and parasol or xylene. The remaining dishes (steps 16-18) are filled with parasol or xylene. The baskets should be left in each dish for approximately 1 to 2 minutes. Sections can be temporarily stored up to 1 hour in the last parasol or xylene storage dishes (step 18). Sections left too long in either solution will become brittle and difficult to mount. One gallon of parasol or xylene will be enough for several thousand grafts.

When the sections in the parasol or xylene dishes are found to be understained with fast green, the baskets can be moved backward through the staining sequence until they are placed in fast green dish for additional staining. The sections are then moved forward through the normal sequence and mounted.

Mounting Equipment, Supplies, and Procedures

Materials needed to mount sections are as follows: microscope slides, cover slips, a medium-sized camel's-hair brush, forceps with pointed tips, absorbent tissues, paper towels, and clove oil. Clove oil is needed only for temporary slides and 1 pint will normally make several thousand slides. For making permanent slides the following are needed: Canadian balsam or other synthetic mounting medium, an eyedropper, and slide labels if slides with frosted ends are not used. Lead weights and a warming table or oven are helpful but not necessary.

For temporary slides, several drops of clove oil are spread in a thin layer over the end of the slide where the sections are to be placed. An eyedropper or a camel's-hair brush works well. Baskets containing the unmounted sections are stored in 95-percent ethanol (step 14, fig. 5) while sections are being placed on the slides. A camel's-hair brush or forceps with pointed tips is used to remove one section at a time from the baskets. Each section should be carefully blotted on absorbent tissue or towel and placed on the area of the slide previously covered with clove oil. Four to six sections from each graft are sufficient for each slide, and only one slide is made from each graft. Several drops of clove oil are placed on top of the sections before the coverslip is lowered. Since only one slide is normally prepared from the sections in each compartment, the foil identifying label can be removed from the basket and positioned on the slide so that one corner of the label is under the edge of the coverslip. One end of the coverslip is positioned near the end of the microscope slide and then is slowly lowered until it completely covers the sections. The sections should be totally immersed in clove oil when the coverslip is completely down. If not, several drops of clove oil should be placed on the edge of the coverslip where it will be absorbed into the dry areas. Temporary mounts are then ready to be examined under a microscope for the presence or absence of incompatibility symptoms. One person can section, stain, and mount 75 to 100 temporary slides in an 8-hour day.

Slides should be systematically arranged as they are completed. Temporary slides can be kept for a week or more before viewing, but a daily examination is better. One check of an entire day's work is more efficient than viewing each slide as it is completed.

After the slides have been examined under the microscope and are no longer needed, they should be

stacked in a single layer on a paper towel. When the paper towel is completely covered with slides, another paper towel is put on top of the first layer, and the operation is repeated until all the used slides are stacked. Stacking used microscope slides in single layers between paper towels keeps cleaning to a minimum. When other slides are to be made, the coverslips and sections are wiped from the slides with absorbent tissues, and both slides and coverslips can usually be reused many times without further cleaning. When work has been completed for the year, the slides and coverslips are either washed and stored for the next year or thrown away.

Permanent slides should be made if microscope preparations are to be kept longer than 1 to 2 weeks. New slides and coverslips are generally used when permanent slides are made. Several drops of balsam or synthetic resin are placed on one end of the slide with an eyedropper or small glass rod and spread evenly across the area where sections are to be placed. Sections are removed from the last parasol or xylene dishes (step 18) and carefully blotted on absorbent tissues before being placed on the slide covered with mounting medium. This work must be done quickly because the mounting medium thickens in the outer surface exposed to the air and causes air bubbles to become trapped under the coverslip when it is lowered over the sections. Before the coverslip is lowered, several additional drops of the mounting medium should be placed on top of the sections. The slide becomes messy if too much is used, but the sections dry out and become worthless if too little is applied. Practice will help determine the correct amount. The coverslip must be lowered slowly so that air bubbles are forced out. Gentle pressure on top of the coverslip will force out many bubbles that have been trapped. Additional resin can be placed along the edge of the coverslip if more medium is required. One small corner of the foil identifying label should be under one edge of the coverslip.

After the coverslips have been placed on the sections, the permanent slides are stored on a flat surface to allow the resin to harden. This takes about 24 hours when a 40°C warming table or oven is used. Or the slides can be stored on paper towels on a flat table or bench top and allowed to harden for 4 to 6 days. Small lead weights (57 g) are placed on top of the coverslips when large grafts or thick sections are covered. The lead weights cause the sections to lie flat on the slide so that most areas of the grafts will appear on one plane when viewed through a microscope. Slides should be handled carefully if it is necessary to view them before the resin has hardened. Permanent slides are more difficult to make than temporary slides and fewer can be completed each day. Until a person becomes familiar with the technique, daily production will be only 30 to 50 slides.

The slides can be stored in slide boxes after about 3 days on a warming table or in an oven, but the slides should not be thoroughly cleaned until 1 to 2 weeks later. This interval allows the mounting medium to harden further and prevents coverslips from coming loose during cleaning. Excess mounting medium should be removed from the edges and upper surface of the coverslips with a razor blade or a tissue moistened with parasol or xylene. Care should be taken to prevent cleaning solvent (xylene or parasol) from getting under the coverslip or it will dissolve the mounting medium. Slides should be identified with permanent labels which contain the appropriate scion clone number and rootstock number.

A low power microscope, 20-40 X, is generally best for classifying Douglas-fir grafts as compatible or incompatible. The scope should not have a mechanical stage if temporary slides are to be examined. Wet slides stick to the stage and are difficult to position correctly. Occasionally, magnifications of 100 X or more are needed.

The mission of the PACIFIC NORTHWEST FOREST AND RANGE EXPERIMENT STATION is to provide the knowledge, technology, and alternatives for present and future protection, management, and use of forest, range, and related environments.

Within this overall mission, the Station conducts and stimulates research to facilitate and to accelerate progress toward the following goals:

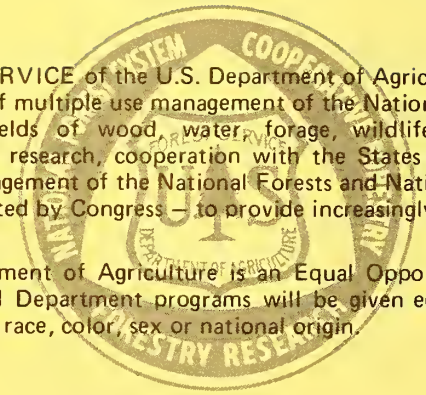
1. Providing safe and efficient technology for inventory, protection, and use of resources.
2. Developing and evaluating alternative methods and levels of resource management.
3. Achieving optimum sustained resource productivity consistent with maintaining a high quality forest environment.

The area of research encompasses Oregon, Washington, Alaska, and, in some cases, California, Hawaii, the Western States, and the Nation. Results of the research are made available promptly. Project headquarters are at:

Fairbanks, Alaska
Juneau, Alaska
Bend, Oregon
Corvallis, Oregon
La Grande, Oregon
Portland, Oregon
Olympia, Washington
Seattle, Washington
Wenatchee, Washington

Mailing address: Pacific Northwest Forest and Range Experiment Station
P.O. Box 3141
Portland, Oregon 97208

GPO 995-147



The FOREST SERVICE of the U.S. Department of Agriculture is dedicated to the principle of multiple use management of the Nation's forest resources for sustained yields of wood, water, forage, wildlife, and recreation. Through forestry research, cooperation with the States and private forest owners, and management of the National Forests and National Grasslands, it strives — as directed by Congress — to provide increasingly greater service to a growing Nation.

The U.S. Department of Agriculture is an Equal Opportunity Employer. Applicants for all Department programs will be given equal consideration without regard to race, color, sex or national origin.